K102286

510(k) Summary
CrAg Lateral Flow Assay

JUL 20 2011

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Owner:
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Prepared: July 18, 2011

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Immunoassay

Regulation: 866.3165

Predicate Device: Immuno-Mycologics' Latex-*Cryptococcus* Antigen Detection System 510(k) # K791382

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum.

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:
Explanation:

Detection of cryptococcal antigen in serum has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (9,14,15). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (16).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (5,6,12,13). Individuals with impaired cell-mediated immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (19),

lymphoproliferative disorders (18), steroid therapy (8), and organ transplantation (7) are at increased risk of cryptococcosis. AIDS accounts for 80-90% of cryptococcal infections (11). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (11), while the incidence of cryptococcosis in other parts of the world, such as Africa, is as high as 30% (3). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (10).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold–conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

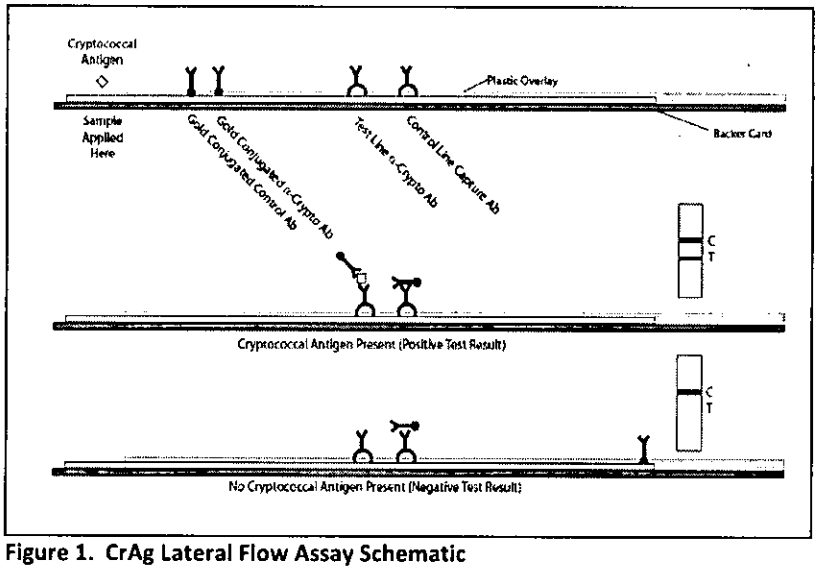

Figure 1. CrAg Lateral Flow Assay Schematic

Technological Characteristics Summary

A comparison between the Cryptococcal Antigen Lateral Flow Immunoassay and the Latex-*Cryptococcus* Antigen Detection System is presented in Table 1.

Table 1. Comparison with Predicate Device

SIMILARITIES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-*Cryptococcus* Antigen Detection System (K791382)
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Sample Matrix	Serum	Serum
Instruments	None	None
Detection Antibody	Anti-cryptococcal antibody	Anti-cryptococcal antibody
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-*Cryptococcus* Antigen Detection System
Intended Use		
Intended Use	Serum only	Serum and CSF
Indication For Use	No differences	No differences
Device Description		
Assay Principle	Lateral flow assay	Latex agglutination
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Serum Pre-Treatment	Serum dilution	Pronase with pronase inhibitor
CSF Pre-Treatment	None	Boil
Detection Antibodies	Gold-conjugated anti-cryptococcal monoclonal antibodies	Latex-conjugated anti-cryptococcal polyclonal antibodies
Storage Requirements	20-25°C	4°C ± 2°

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Serum:

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection

System with cryptococcal antigen at four concentrations: Negative, high negative (C_S), low positive (near C_{95}), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Serum Repeatability at 3 Different Sites

	Serum							
Sample	1		2		3		4	
	Med. Pos		Low Pos		High Neg		Neg	
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

B. Analytical Sensitivity (lower limits of the assay/analytical cut-off)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in Lateral Flow (LF) Specimen Diluent, according to EP12-A2. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml.

C. Analytical Specificity (cross-reactivity)

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. A total of 118 serum specimens were tested in triplicate. Percent positive was determined for each condition (Table 3).

Table 3. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)

Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/ml.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/ml), antigens from *Paracoccidiodes brasiliensis* exhibitied some cross-reactivity. Antigens from *Aspergillus terreus, Aspergillus fumigatus, Aspergillus niger,* and *Aspergillus flavus* did not exhibit cross-reactivity.

Interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip.

The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Cryptococcal EIA positive specimens and 5 Cryptococcal EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis, Candida tropicalis, Candida parapsidosis, Candida krusei, Candida glabrata, Cladosporium trichoides, Neisseria meningitidis, Salmonella typhi, Pneumocystis carinii, Trichosporon beigelii, Zygomycetes*, ANA+, HAV, HCV, *Staph*, and *Strep*.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations

between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparison

Method Comparison: Cryptococcal Antigen EIA

The CrAg Lateral Flow Assay was compared to a Cryptococcal Antigen EIA, which detects cryptococcal antigens in serum.

A panel of 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing, using the Cryptococcal Antigen EIA, were collected and stored frozen until method comparison studies were performed. Specimens were then thawed and tested concurrently in the CrAg LFA and in a latex agglutination test [See "Predicate Method Comparison – Latex-Cryptococcal Antigen Detection System (K791382)]. The resulting data is presented in a 2x2 contingency table (Table 4). The percent agreement positive, percent agreement negative, and 95% confidence interval are also presented (Table 5).

Table 4. Serum 2x2 Contingency Table

	EIA (+)	EIA (-)
CrAg Lat Flow (+)	96	7
CrAg Lat Flow (-)	0	94

Table 5. Serum Statistical Analysis

		95% CI
% Agreement Positive	100%	96-100%
% Agreement Negative	93%	86-97%

Other Method Comparison – Culture/India Ink (Gold Standards)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected in included in Tables 6 and 7 below:

Table 6. Serum 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA	Positive	91	0

Assay	Negative	0	123

Table 7. Serum Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	96.0%-100%
Specificity	100%	97.0%-100%

Predicat Device Comparison – Latex-Cryptococcal Antigen Detection System (K791382)

Serum Specimens:

A panel of 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing, using a Cryptococcal Antigen EIA, were collected and stored frozen until method comparison studies were performed. Of the 197 specimens, 96 were positive and 101 were negative by the EIA. All specimens were analyzed, according to EP12-A2, concurrently in the CrAg Lateral Flow Assay and in the IMMY Latex-*Cryptococcus* Antigen Detection System to ensure the data was not affected by the freeze-thaw cycle. Each specimen was tested at IMMY in duplicate in both tests according to each test's package insert. The data is presented in a 2x2 contingency table (Table 8). The percent agreement positive, percent agreement negative, and 95% confidence interval are also presented (Table 9).

Table 8. Serum 2x2 Contingency Table: Latex Agglutination

	Latex (+)	Latex (-)
CrAg LFA (+)	101	2
CrAg LFA (-)	0	94

Table 9. Serum Statistical Analysis: Latex Agglutination

		95% CI
% Agreement Positive	100%	96-100%
% Agreement Negative	98%	93-99%

Semi-Quantitative Serum

Sixty-two serum specimens that tested positive by the IMMY Latex-*Cryptococcus* Antigen Detection System during the qualitative method comparison were stored frozen (-80° C) then analyzed in the CrAg Lateral Flow Assay (LFA) to determine the specimens' titers (Semi-quantitative analysis). Concurrently, the specimens were tested in the IMMY Latex-*Cryptococcus* Antigen Detection System (LA) to determine the latex titer for each specimen. The entire panel was tested at IMMY according to each test's package insert. Cryptococcus Antigen Latex titer versus CrAg Lateral Flow Test titer was plotted and regression analysis performed. The data show a strong correlation between the two tests ($R^2 = 0.905$).

Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Immuno-Mycologics, Inc
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology Place
Norman, OK 73071

JUL 20 2011

Re: k102286

Trade/Device Name:	CrAg Lateral Flow Assay
Regulation Number:	21CFR §866.3165
Regulation Name:	*Crptococcus neoformans* serological reagents.
Regulatory Class:	Class II
Product Code:	GMD
Dated:	July 18, 2011
Received:	July 19, 2011

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050. This letter will allow you to begin marketing your device as described in your Section

510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number (if known): K102286

Device Name: CrAg Lateral Flow Assay

Indications for Use:

The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum.

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Prescription Use ✓ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Freddie L. Poole

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k): K102286